

WHAT IS CLAIMED IS:

1. An isolated nucleic acid segment comprising a δ -endotoxin gene encoding a Cry* polypeptide selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:100, and SEQ ID NO:108.
2. The polynucleotide of claim 1, further defined as comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:99, SEQ ID NO:99, and SEQ ID NO:107.
3. The polynucleotide of claim 1, further characterized as DNA, cDNA, rRNA, or mRNA.

4. A nucleic acid segment of from about 1800 to about 18,000 nucleotides in length comprising a gene encoding a modified Cry3* δ -endotoxin polypeptide having improved insect activity or enhanced insect specificity against a target insect when compared to the unmodified Cry3 polypeptide.
5. The nucleic acid segment of claim 4, wherein said nucleic acid segment is from about 2000 to about 10,000 nucleotides in length.
6. The nucleic acid segment of claim 5, wherein said nucleic acid segment is from about 3000 to about 8,000 nucleotides in length.
7. The nucleic acid segment of claim 4, wherein said gene is operably linked to a promoter, said promoter expressing said gene.
8. The nucleic acid segment of claim 4, comprised within a vector.
9. The nucleic acid segment of claim 4, comprised within a transgenic plant.
10. The nucleic acid segment of claim 4, wherein said modified Cry3* crystal protein is obtained by random or site-specific mutagenesis of the nucleic acid segment which encodes it.

11. A nucleic acid segment of from about 1800 to about 18,000 nucleotides in length comprising a *cry3** gene encoding a modified Cry3* crystal protein, wherein said modified Cry3* crystal protein has improved insecticidal activity or specificity when compared to a native unmodified Cry3 crystal protein.
12. The nucleic acid segment of claim 11, wherein said modified Cry3* crystal protein is obtained by random or site-specific mutagenesis of the nucleic acid segment which encodes it.
13. The nucleic acid segment of claim 12, wherein said mutagenesis results in one or more nucleotide substitutions, deletions, or insertions.
14. The nucleic acid segment of claim 13, wherein said mutagenesis results in one or more amino acid substitutions, deletions, or insertions in the encoded Cry3* protein.
15. The nucleic acid segment claim 11, wherein said gene encodes a Cry3Bb* crystal protein selected from the group consisting of Cry3Bb-60, Cry3Bb.11221, Cry3Bb.11222, Cry3Bb.11223, Cry3Bb.11224, Cry3Bb.11225, Cry3Bb.11226, Cry3Bb.11227, Cry3Bb.11228, Cry3Bb.11229, Cry3Bb.11230, Cry3Bb.11231, Cry3Bb.11232, Cry3Bb.11233, Cry3Bb.11234, Cry3Bb.11235, Cry3Bb.11236, Cry3Bb.11237, Cry3Bb.11238, Cry3Bb.11239, Cry3Bb.11241, Cry3Bb.11242, Cry3Bb.11032, Cry3Bb.11035, Cry3Bb.11036, Cry3Bb.11046, Cry3Bb.11048, Cry3Bb.11051, Cry3Bb.11057, Cry3Bb.11058, Cry3Bb.11081, Cry3Bb.11082, Cry3Bb.11083, Cry3Bb.11084, Cry3Bb.11095, and Cry3Bb.11098.

16. The nucleic acid segment of claim 11, wherein said *cry3** gene is further defined as a *cry3A**, *cry3B**, or *cry3C** gene.

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17. The nucleic acid segment of claim 16, wherein said *cry3B** gene is further defined as a *cry3Bb** gene.

10 18. The nucleic acid segment of claim 17, wherein said *cry3Bb** gene is further defined as a *cry3Bb-60*, *cry3Bb.11221*, *cry3Bb.11222*, *cry3Bb.11223*, *cry3Bb.11224*, *cry3Bb.11225*, *cry3Bb.11226*, *cry3Bb.11227*, *cry3Bb.11228*, *cry3Bb.11229*, *cry3Bb.11230*, *cry3Bb.11231*, *cry3Bb.11232*, *cry3Bb.11233*, *cry3Bb.11234*, *cry3Bb.11235*, *cry3Bb.11236*, *cry3Bb.11237*, *cry3Bb.11238*, *cry3Bb.11239*,
15 *cry3Bb.11241*, *cry3Bb.11242*, *cry3Bb.11032*, *cry3Bb.11035*, *cry3Bb.11036*, *cry3Bb.11046*, *cry3Bb.11048*, *cry3Bb.11051*, *cry3Bb.11057*, *cry3Bb.11058*, *cry3Bb.11081*, *cry3Bb.11082*, *cry3Bb.11083*, *cry3Bb.11084*, *cry3Bb.11095* or *cry3Bb.11098* gene.

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19. A method of preparing a modified Cry3* polypeptide having improved insecticidal activity or specificity, comprising the steps of:

25 (a) providing a nucleic acid segment comprising a gene which encodes a Cry3* polypeptide, wherein said gene is operably linked to a promoter which expresses said gene;

(b) introducing said nucleic acid segment into a vector;

30 (c) transforming a host cell with said vector; and

(d) culturing said host cell under conditions effective to allow expression of the modified Cry3* polypeptide.

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20. The method of claim 19, wherein said gene encodes a modified Cry3A*, Cry3B*, or Cry3C* polypeptide.

10 21. The method of claim 19, wherein said host cell is a plant, animal, viral, fungal, archaeobacterial, eubacterial, or cyanobacterial host cell.

15 22. The method of claim 19, wherein said gene comprises a *cry3A**, *cry3B**, or *cry3C** gene.

23. The method of claim 22, wherein said *cry3B** gene is selected from the group consisting of a *cry3Bb-60*, *cry3Bb.11221*, *cry3Bb.11222*, *cry3Bb.11223*,
20 *cry3Bb.11224*, *cry3Bb.11225*, *cry3Bb.11226*, *cry3Bb.11227*, *cry3Bb.11228*,
cry3Bb.11229, *cry3Bb.11230*, *cry3Bb.11231*, *cry3Bb.11232*, *cry3Bb.11233*,
cry3Bb.11234, *cry3Bb.11235*, *cry3Bb.11236*, *cry3Bb.11237*, *cry3Bb.11238*,
cry3Bb.11239, *cry3Bb.11241*, *cry3Bb.11242*, *cry3Bb.11032*, *cry3Bb.11035*,
cry3Bb.11036, *cry3Bb.11046*, *cry3Bb.11048*, *cry3Bb.11051*, *cry3Bb.11057*,
25 *cry3Bb.11058*, *cry3Bb.11081*, *cry3Bb.11082*, *cry3Bb.11083*, *cry3Bb.11084*,
cry3Bb.11095, and *cry3Bb.11098*.

24. A vector comprising a nucleic acid segment encoding a *B. thuringiensis* Cry3*
30 polypeptide, wherein said polypeptide has at least one modified amino acid

sequence in one or more domains of said polypeptide, relative to a wild-type unmodified Cry3 polypeptide; and further wherein said modified amino acid sequence confers to said polypeptide an insecticidal activity greater than, or an insecticidal specificity broader than, that of said wild-type unmodified Cry3 polypeptide.

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25. The vector of claim 24, wherein said Cry3* polypeptide is selected from the group consisting of Cry3Bb-60, Cry3Bb.11221, Cry3Bb.11222, Cry3Bb.11223, Cry3Bb.11224, Cry3Bb.11225, Cry3Bb.11226, Cry3Bb.11227, Cry3Bb.11228, Cry3Bb.11229, Cry3Bb.11230, Cry3Bb.11231, Cry3Bb.11232, Cry3Bb.11233, Cry3Bb.11234, Cry3Bb.11235, Cry3Bb.11236, Cry3Bb.11237, Cry3Bb.11238, Cry3Bb.11239, Cry3Bb.11241, Cry3Bb.11242, Cry3Bb.11032, Cry3Bb.11035, Cry3Bb.11036, Cry3Bb.11046, Cry3Bb.11048, Cry3Bb.11051, Cry3Bb.11057, Cry3Bb.11058, Cry3Bb.11081, Cry3Bb.11082, Cry3Bb.11083, Cry3Bb.11084, Cry3Bb.11095, and Cry3Bb.11098.

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26. The vector of claim 25, wherein said Cry3* polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:100, and SEQ ID NO:108.

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27. The vector of claim 26, wherein said Cry3* polypeptide is encoded by a nucleic acid segment selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:99, and SEQ ID NO:107.
28. The vector of claim 24, transformed and replicated in a prokaryotic or eukaryotic host.
29. The vector of claim 28, comprised within a plant cell.
30. The vector of claim 24, wherein said polypeptide is encoded by a nucleic acid segment contained within a plasmid selected from the group consisting of pEG1707, pEG1708, pEG1709, pEG1710, pEG1711, pEG1712, pEG1713, pEG1714, pEG1715, pEG1716, pEG1717, pEG1718, pEG1719, pEG1720, pEG1721, pEG1722, pEG1723, pEG1724, pEG1725, pEG1726, pEG1727, pEG1041, pEG1046, pEG1047, pEG1052, pEG1054, pEG1057, pEG1062, pEG1063, pEG1084, pEG1085, pEG1086, pEG1087, pEG1095, and pEG1098.
31. The vector of claim 24, further defined as a cosmid, plasmid, phagemid, baculovirus, artificial chromosome, or viral vector.

32. A vector comprising the nucleic acid segment of claim 1.
- 5 33. A transformed host cell which expresses the polynucleotide of claim 1.
34. A method of preparing a Coleopteran-resistant transgenic plant, comprising the steps of:
- 10 (a) obtaining a nucleic acid segment comprising a gene which encodes a modified Cry3* polypeptide, wherein said gene is operably linked to a promoter which expresses said gene;
- 15 (b) introducing said nucleic acid segment into a vector;
- (c) transforming a plant cell with said vector; and
- (d) generating from said plant cell a transgenic plant which expresses said modified Cry3* polypeptide.
- 20 35. The method of claim 34, wherein said gene comprises a δ -endotoxin gene encoding a Cry3* polypeptide selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID
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NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:100, and
SEQ ID NO:108.